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REVIEW

Interactions between the Genetic Programme and Environmental Influences in the Perinatal Critical Period

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ABSTRACT—In certain systemes of the organism the reinforcement of genetically loosely determined traits is necessary and it is realised under the effect of the actual (new) inner and outer milieu perinatally, mostly after birth. In this period the sexual, hormonal, enzyme, and behavioral imprinting are taking place and this is the time of closing of the self-nonself discrimination. The program stored in the genes is selected and activated in that time, what makes the effect of perinatal adjusting (imprinting) practically equal with the genetic determination. Because of this, rather difficult is to differentiate between the effects of imprinting and genes. In the critical period of adjusting (imprinting) chemical materials disparate from the normal ones, (however effective and able to bind to receptors) and faulty actions (in the case of behavioural imprinting) can cause faulty imprinting, whose effects are measurable for life. The possibility (and importance) of the faulty imprintings is growing nowadays.

INTRODUCTION

The morphological and functional traits of living beings, including man, are essentially determined by the genes. At the lower levels of phylogenesis, genetic determination leaves little free play for environmental influences, but survival presupposes adaptation to the environment also in lower organisms. Therefore those gene-automatons, which resist adverse environmental effects and adapt themselves readily to environmental changes, have a better chance for survival and reproduction than the less adaptable ones. At the higher levels of phylogenesis, environmental factors play a still more important role, since the higher an organism, the lesser are its chances for a simple "yes" or "no" answer to environmental signals (factors). It follows that, in higher organisms, the environmental factors influence the expression of genetic traits not only via selection in the progeny generations, but also by direct effect. Environmental influence is greatest at the level of the human beings, for biological and social factors have a practically equivalent impact on human development by interdependent effects.

The genetic and social factors determining human development are complementary, and mutually influence each other. Certain environmental influences take effect only in given genetic conditions and vice versa, certain genetic programmes can run only if the environment favours their operation. The purpose of this review is to throw more light on the interdependence of genetic and environmental factors, by analysis of four selected aspects of imprinting which show that the impact of the environment on ontogenetic development may be occasionally as great as that of genetic determination itself.

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THE FOUR FORMS OF IMPPINTING

Imprinting of the sex

The structural aspects of sex are extraordinarily intricate in all mammals, including man. Although the sex of an individual is essentially determined by the sex chromosomes (chromosomal sex), the differentiation of the male or female sex characteristics proceeds over many steps of regulation.

Antigen H-Y, which depends on the Y chromosome, brings about the differentiation of the originally neutral gonads to male sex organs, whereas in absence of the Y chromosome, the X chromosome accunts for differentiation to female sex organs. The testosterone produced by the male gonad, and the Müllerian duct inhibitor promote, by joint effect, the development of the Wolffian ducts and simultaneously the atrophy of the Müllerian ducts. As differentiation proceeds, the phallus and the urogenital sinus become the penis and scrotum under the influence of testosterone, whereas in the latter's absence they become the clitoris and vulva.

These processes take place during embryonic or foetal development, under the control of the genetic programme. Any disturbance in the programme results in a faulty functioning of the system.

The formation of the male or female sex organs is followed by the establishement of sexual behaviour. Males and females equally have their own characteristic behavioural traits. The copulative behaviour of the male rat is responded by the female by a submissive lordosis reflex, which makes possible the immission of the penis. These or analogous behavioural patterns are shown by the adult males and females of all mammalian species, and cannot be extinguished by treatment with hormones of the opposite sex. The normal female rat shown, under the influence of oestrogenic hormones, the lordosis reflex on approach of a male prepared for copulation, and the testosterone-treated male will mount the female and copulate as outlined above. Treatment of the female with testosterone and of the male with oestrogen will diminish, but not alter, the expressions of sexual behaviour [1]. However, if treatment with hormones of the opposite sex is carried out in the perinatal period, a radical change of the sexual behaviour will follow in adulthood.

Female rats treated with testosterone in the perinatal period (from within a few days before birth to 10 days post partum) show no lordosis reflex on approach of the male in adulthood, not even under the influence of exogenous oestrogen. Similarly, males castrated perinatally will not approach the submissive females, and remain refractory to exogenous testosterone. However, males castrated perinatally but given simultaneously testosterone will respond to it in adulthood like normal controls. It follows that the pattern of adult sexual behaviour and of the adult response to specific sex hormones becomes established in the perinatal period [2].

Female rats postnatally treated with testosterone failed to develop the characteristic cyclicity of sexual function after sexual maturation, and so did the male rats postnatally treated with oestrogen. Since postnatal oestrogen overdose had the same effect on female rats as testosterone (which became itself aromatized to oestrogen), it was concluded that the development of the male or female patterns of sexual behaviour depended not so much on the quality as the guantity of the sexual hormone dose [2–6].

Oestrogen develops action at receptor level, by programming the activity of the hypothalamic cells responsible for the production of the gonadotropin releasing hormone (GnRH). This programme controls—after being transferred to the pituitary —the cyclicity or continuity of sexual hormone production. Thus, while the gonadal steroid hormone pruduction is under hypophyseal control, the trend of hypophyseal gonadotropin production is induced by perinatal steroid hormone influence. The possibility for induction is limited to a brief critical period, and fails to take effect either before or after it. Testosterone, for example, is also present in the organism before the critical period, its influence being a prerequisite of the development of the gonadal ducts and of the external genital organs as well, but it develops action at the cerebral receptorial level only later, around birth. During the critical period the gonadal receptors are responsive not only to endogenous, but also the exogenous hormonal influence, which may, in part of the cases, account for a faulty development, whose consequences become expressed only during sexual maturation. Apart from exogenous sex hormones also other, apparently indifferent substances (e.g. drugs) may alter the endogenous sex hormone level [7] and, ultimately, the sexual behaviour.

Dörner *et al.* [8] believe that human homo- and bisexual traits, too, stem from disturbances in sex differentation, on the ground that a history of

maternal stress during pregnancy has been considerably more frequent among homo- and bisexual than heterosexual groups of humans. This implication does not, naturally, exclude the genetic determination of sex, because part of the mothers subject to stress during pregnancy deliver sexually normal children, and vice versa: part of the homo- or bisexuals are issues of thoroughly normal pregnancies.

While in higher organisms the environmental influences (except drastic intrauterine interventions, such as hormone treatment during pregnancy) alter only the sexual behaviour, in lower organisms they may alter the chromosomal sex. For example, treatment of the female larval stage (XX) of the fish Orizias latipes with androgenic hormone results in the development of an apparently normal male fish. However, mating of that male to a female gives rise exclusively to female progeny. Treatment of a male Orizias latipes larva (XY) with oestrogen results in the development of an apparently normal female which may, on mating to a male, produce both male (YY) and female (XY) progeny. The larval stages of certain amphibians, too, may change their chromosomal sex in response to treatment with the key hormone of the opposite sex. Moreover, environmental influences other than hormonal may change the chromosomal sex of lower organisms. Frog eggs develop to males at a supraoptimal water temperature, and to females at a suboptimal temperature. The eggs of certain lizards invariably develop to females at hatching temperatures less than 26°C, and to males above 26°C. Two tortoise species have shown a similar (although reverse) tendency of temperature impact on sexual development. Apart from ambient temperature, the environmental Ca^{2+} level may also play a role in the development of the same egg to a male or female embyro [2].

The foregoing observations strongly suggest that *the genetic determination of sex is only virtual or partial, because environmental factors play a decisive role in sexual development.*

Hormonal and enzyme imprinting

The functioning of the endocrine system presupposes the availability of hormones and receptors. The endocrine organs produce hormones, and some receptors are able to receive signals already during intrauterine life. However, prenatal and adult receptorial activities are not comparable, for most receptors are still immature around birth. Receptor maturation being an integral part of cell differentiation, maturity means the establishment of the hormone binding and hormon transfer capacities characteristic of the adult. However, hormone receptors require presence of the adequate hormone for maturation [9]. If, for example, the production of thyrotropic hormone (TSH) is depressed postnatally by thyroxin treatment, the TSH receptors fail to develop normally, and account for a reduced thyroidic response to TSH in adulthood [10]. The receptor may also be damaged by excessive presence of the adequate hormone in the critical period, which may give rise either to an increased, or to a decreased receptor activity in adulthood, depending on the species of the animal and the degree of maturity of the receptor [11].

The above experimental observations equally apply to polypeptide, amino acid and steroid hormones. The phenomenon has been termed generally as *hormonal imprinting*. It was also shown that presence during the critical period of receptor maturation of molecules structurally similar to the adequate hormone (e.g. hormone analogons or related hormones) and therefore capable of binding to its receptor, biases receptor maturation and gives rise to faulty imprinting [12] either in the positive or in the negative sense. For example, treatment of neonatal rats with a gonadotropin over-dose on single to several occasions accounted for a considerable reduction of adult response to exogenous thyrotropin and the endogenous TSH as well [13]. The cause of this phenomenon was obviously the fact that since gonadotropin and thyrotropin have a common alpha subunit, and some sequences overlap also in their beta subunits, the hormonal overlap, which is functionally irrelevant in adulthood, had given rise to a serious receptor damage in the critical perinatal period. Perinatal overlap of vasopressin-oxytocin, steroid-steroid, etc. had similar consequences in experimental condition [14]. As already pointed out above, the adequate hormone also

accounts for receptor damage in the critical perinatal period, if present in excess. Imprinting depends not only on the age of the individual, but also on the stage of cell development. It follows that although all receptor-carrying cells become imprinted in the perinatal period, those cells, which preserve developmental potentials later in life (e.g. undifferentiated or cell-forming cells) are available for imprinting also in adulthod [15, 16]. For example, hepatocytes newly formed during liver regeneration experience a re-imprinting, which is as durable as the original perinatal imprinting [17, 18].

Evidence of re-imprinting of re-differentiating cells, which had lost the "memory" of original imprinting by de-differentiation, was obtained from experiments on cell lines [12]. These experiments demonstrated the spread of imprinting from imprinted to not imprinted cells by direct cell-cell contact [19].

The hormones imprint in the perinatal period not only their own receptors, but also the enzymes responsible for hormone splitting [20]. For example, perinatal imprinting by a sexual steroid, namely testosterone, has a life-long impact on the functioning of the microsomal enzyme system.

It follows from the foregoing considerations that *although the receptor system responsible for recognition of the hormone and mediation of the hormonal signal is genetically encoded,* and no receptor can arise without encoding, *it requires interaction with an appropriate concentration of the adequate hormone at the appropriate time,* or else no imprinting takes place and the receptor fails to mature.

Cross-imprinting can not infrequently occur in the critical period of receptor maturation. For example, sexual steroids may imprint glucocorticoid receptors (and vice versa) in the perinatal period [14, 21]. Similarly, gonadotropin and thyrotropin may be simultaneously present and imprint one another's receptors. To obviate the risk of serious damages by cross-imprinting, the perinatal hormone peaks appear at different time intervals, thus minor crossing-overs do not interfere with the normal course of imprinting.

Perinatal imprinting achieves *the establishment of the receptor-hormone relationship characteristic of adulthood.* However, non-physiological materials capable of receptor-level action may also give rise to imprinting in the perinatal period. Among others benzo-a-pyrene, an aromatic hydrocarbon of steroid-like structure, altered the binding capacity of rat steroid receptors in adulthood [22–24] regardless whether it was administered *prenatally neonatally* or at *three or six weeks of age.*

Treatment of lactating rat females with diazepam altered the selective ligand binding capacity to the suckling rats in adulthood, and also accounted for certain negative behavioural changes [25]. Neonatal treatment with digoxin or ouabain changed the adult binding capacity of the myocardial ouabain receptor [14]. Since, however, like steroids, digoxin has a sterane structure, perinatal steroid treatment, too, had a certain influence on the ouabain receptor [40].

Perinatal treatment with caffeine [26] accounted for a considerable increase in adult gastric ulcer susceptibility. Prenatal alcohol administration altered alcohol sensitivity in adulthood and increased tolerance to druge eliciting cross-tolerance to alcohol in man, as e.g. phenobarbital and diazapam. Moreover, *rats and pigs treated with alcohol in utero consumed in adulthood considerably more alcohol than those not exposed to it prenatally.* [*27, 29*].

By analogy of hormonal imprinting, the enzymes may be influenced by their substrates in the perinatal period. For example, neonatal exposure to benzo-a-pyrene or methylcholanthrene has a life-long impact on the hepatic microsomal enzyme system [30].

Imprinting by drugs (chemicals) depends not so much on the age of the individual, as on the stage of development of the target cells. Adult patients chronically treated with digoxin showed a considerable change in erythrocytic digoxin binding relative to the untreated controls [31].

Since benzo-a-pyrene and digoxin develop action at receptor level, their effect on the receptors resembles that of hormonal imprinting. However, it appears that imprinting can take place also at levels other than receptorial, for the actions of caffeine and alcohol are clearly receptor-unrelated. The underlying mechanism is in all probability the neonatal adjustment of the enzyme

system responsible for the splitting of these materials, [32, 33], as is the case with the influence of neonatal exposure to steroid hormones or benzo-a-pyrene on the functioning of the microsomal enzyme system in adulthood [34].

It follows that *imprinting is an issue of interaction between the ligand and its binding site, regardless whether a receptor—hormone relationship or an enzyme—substrate relationship is involved.* The main point is the establishment of the final relationship, which may be normal or abnormal, depending on the nature of molecules which appear in the critical period.

Behavioural imprinting

The conception of the perinatal behavioural imprinting of animals was originated by Lorenz and has since been generally adopted by animal scientists. The newly hatched duckling would follow its mother, but in absence of the mother the following reflex may be fixed on an object (e.g. a ball) or a human being, whose movements the duckling perceives visually during the critical period of behavioural imprinting. Thus the duckling develops a certain "sentimental" attachment to the imprinting object, whatever the latter's nature. The impact of this attachment lasts for life-time. Imprinting for the recognition of species or breed mates, and of the opposite sex or even an object as potential sexual partner also takes in the critical period, which is definitive and relatively short, but does not terminate abruptly [35, 36].

The song repertory of a finch includes about 20 tune patterns, of which 17 are possessed by heredity, but three are sung only by those finches, which had been reared by the parent. Artificially reared finches either fail to sing the three patterns in question, or present others lasting for similar time [37, 38]. This observation again seems to substantiate the implication that, although the behavioural patterns are genetically encoded, they require amplification by environmental influence for coming into display. Finches exposed during rearing to hearing the songs of many other birds would learn only the three tune patterns in question. It follows that *the learning potential is itself genetically encoded, and limits the selection of information to given range*, while it simultaneously presupposes that the patterns to be learned are present in the critical period of imprinting. *Thus the behavioural patterns are encoded in an open genetic preogramme, which requires a key stimulus for activation and fixing.* For example the picking reflex of the chick is activated by the sight of the stick-like legs of itself or its mates, but matchsticks are also suitable for activation [39].

Harlow demonstrated in his famous experiment that behavioural imprinting also occurs in mammals as high as apes. While a baby monkey reared by its mother developed to a social being, the one maintained on a haired phantom mother developed unsocial traits, and the third maintained on a wire phantom developed frankly antisocial patterns of behaviour [40]. Observations on humans have affirmed that imprinting plays a decisive role in the development of the human individual, too. The environmental factors acting in the postnatal period, namely the maternal influence, have a life-long impact on the newborn baby. It appears that the period of imprinting lasts considerably longer in humans than in animals, for it begins with prenatal adjustment to maternal influences (e.g. maternal heart function) and continues for a relatively long time after birth. It has been suggested that the foetus can perceive the tones of the mother's voice already in utero [39]. This is not surprising, if it is taken into consideration that vocal stimuli also act on the developing duck embryo inside the egg, although its connection with the maternal body is much less intimate than that of the human embryo.

Although behavioural imprinting seems to depend entirely on the nervous system, it is associated to a considerable degree with simple chemical components. Among other traits food preference, too, is also developed in the perinatal period. Experiments with tortoises have shown that the animals prefer those foods in adulthood, which had been available in the critical period of perinatal imprinting. A single "meal" may be sufficient for imprinting food preference. The chemical rather than nervous basis of that aspect of imprinting is shown by the experimental fact that perinatal fat and cholesterol diet had a demonstrable impact on cholesterol homeostasis in adulthood [39].

Thus the conclusion lies close at hand that

although the elements of the behavioural patterns are genetically encoded, the genetic programme is open and the patterns require environmental influence for amplification in the overwhelming majority of the cases.

Certain elements of imprinting also persist in adulthood. The birds parents "learn" the characteristics of their offspring at the first hatching, and will not accept different traits in the subsequent offspring generations. In mammals, imprinting for maternal behaviour is induced by olfactory stimuli. Avian and mammalian maternal imprinting is equally induced by the birth of the first offspring, since it cannot, by nature, arise before the reproductive age. Maternal imprinting, too, is limited to a relatively brief period, which terminates practically with the establishment of the maternal behavioural patterns [38, 39].

Behavioural imprinting persists as a rule latently and becomes expressed only in response to the specific stimulus. Male birds imprinted for another avian species will prefer sexual partners of the foreign species to whereas female birds do not develop such preference. If, however, the females imprinted for a foreign species receive testosterone later in life, they will preferentially approach —with male behavioural patterns—the foreign females. This observation substantiates the existence of imprinting and the necessaity of its association with a hormonal stimulus as well.

Immunological imprinting: differentiation of "self" from "non self"

The preservation of the integrity of an organism is the responsibility of the immune system, whose cells destroy, either directly or by immune secretions, the materials or structures recognized as "nonself" (foreign) but do not in any way influence the structurally similar entities recognized as "self". The question arises, how the immune system can "tell" self from foreign? The recognition of "foreign" is possible only relative to "self", because structures which represent "self" for one organism are "foreign" to the other. Obviously, several chances are available for the immune cells during their formation. One possible explanation is that each organism forms for itself a special set of immune cells which do not produce antibodies to any material or structure (of antigenic potential) present in the organism itself, but do produce them against those which either enter into the organism from the outside world, or behave inside it as "foreign". The alternative explanation is that the immune cells are capable to act either directly or indirectly by immune secretions, against all materials or structures which ever have, or ever will have, behaved as a potential antigen. However, in that case the system is obliged to "learn" the differentiation of "self" from "non-self".

All nucleated cells posses markers (class I MHC) which are characteristic of the given micro- or macroorganism. with these markers is associated the capability of differentiating "self" from "non-self" [41]. The markers (antigens) present in the organism during the development of the immune system account for suppression of all clones which were capable of producing antibodies to them, and exclude threby the production of auto-antibodies (antibodies to "self"), but do not interfere with the proliferation and the immunological potential of the other clones. However, the cells of the suppressed—"forbidden"—clones survive, and their inhibition depends of the continuous presence of the antigen (marker), because the autoantibody producing capacity immediately returns in absence of the marker.

The suppression of the self-compartment of the immune system is not a singular event: it takes place continuously. Certain antigens suppress the adequate immune clones already in prenatal life, while others act on these only in the early postnatal period. Since, however, the perinatal stage of suppression terminates by the end of the early postnatal period, the auto-antigens appearing later in life (ocular lens, male gametes etc.) are neutralized by other protective mechanisms.

It follows that the operation of *the system accounting for differentiation between "self" and "non-self" is also associated with intrauterine or perinatal imprinting, and is based on a negative immune "memory"*, which functions excusively in presence of the imprinter.

INTERRELATIONSHIPS BETWEEN GENETIC PROGRAMME AND ENVIRONMENT DURING ONTOGENETIC DEVELOPMENT

The foregoing considerations on the four main aspects of imprinting (more are practically not known) strongly suggest the relativity of genetic determination in certain cases. The genes themselves only determine—or rather de-limit—a certain range of potentials, their role being the limitation rather than the unfolding of the totality of possibilities available. Thus *the genes offer essentially a set of choices, of which either the environmental influences operative at a given time will select the actual trend of development, and the available genes determine the potential place and time for an actually suitable selection.* If the actual chance is missed, only some other trends of development are possible, which may result in an inadequate function. It follows the *the choice from among the possibilities offered by the genes is deterministic rather than chance-dependent,* yet, since the genetic programme is plastic, it does require the collaboration of imprinting (environmental influence).

The nature of the systems brought into operation by imprinting also deserves consideration. The endocrine, nervous and immune systems are essentially recognition systems, in which the interrelationships between the elements of the system, and between the system as a whole and its environment play a decisive role. The imprinting of sex is partly outside this category, for chromosomal sex is endogenously determined, partly satisfies the criteria of a recognition system, inasmuch as it involves receptor-level events and presupposes a choice between two possibilities (male or female) at all levels involved in the expression of one or the other sex. The nervous system recognized presence of a male or female gonad by receiving the adequate hormonal signal. In many event, sex differentiation presents the most striking example of the plasticity of the genetic programme, for the phenotypic expression of sex may considerably deviate from, or even be opposite to, the chromosomal sex (Morris's disease: testicular feminization). The same extent of plasticity would, in other systems, imply such extreme changes as for example development of the liver to a pancreas, or vice versa. Since, however, such changes have been unprecendented, there is reason to postulate that exclusively recognition systems are endowed with those potentials, which permit selection from a wide range of possible choices by means of imprinting.

The protein patterns encoded in the gene may be similar in unicellulars and single human cells. The *specific difference* between unicellular organism and a human liver, nerve etc. cell is associated with the intra-genomic localization and regulation of the protein patterns, i.e. on the given *genetic programme.* The normal development of an organism presupposes the operation of an intact genetic programme. Regulatory or structural errors in the genome account for a faulty development. These circumstances presuppose rather than exclude the collaboration of environmental factors, i.e. of interactions at intra- and/or intercellular level as well as between the organism and its environment. During ontogenetic development, the activation or termination of a programme depends in most cases on the product of another cell which, although itself an issue of a programme, acts on the target cell as an environmental factor.

The "other cell" or its product can act on the target cell only at a time when the target cell's programme is available for activation. If activation is missing, the target cell fails to develop (or to function), and this failure will have a major impact on the signal emitting cell as well as on a third cell which would have depended on the target cell for environmental influence. It follows that the normal course of ontogenetic development, too presupposes the interaction of gene level events and environmental factors (activable genetic programmes and key stimuli), and although the environment could never activate a programme not carried (permitted) by the genes, the genetic information requires the presence (collaboration) of an environment for expression. In view of this it is not surprising that the recognition systems of the organism, which are responsible for contact with the environment, depend to a greater degree than other systems (cells) on environmental influence for the unfolding of their genetically programmed

structure and function.

The "tuning" of signal recognition systems

If recognition systems are interpreted in terms of the technical-cybernetical nomenclature, one may characterize the information stored in the genes as the structure which determined the capacity of a receiver, and the number of the signal emitters it can cover, but does not determine the quality of signal reception. The quality of reception depends on tuning, more precisely on fine tuning, which requires a special intervention. In this sense, sexual and immunological imprinting depend entirely on external intervention (direction-finding of sex differentiation, resp. suppression of cells against autoantigens), whereas behavioural and hormonal imprinting (hormone receptor formation) evolve exclusively by fine tuning.

The normal functioning of an organism presupposes the normal operation of all its component organs and/or cells. All cells of an organism carry the same genetic information, but each realizes a different part of the genetic programme during ontogenetic development. Cell differentiation accounts for the morphological and functional dissimilarity (specialization) of the cells integrated into the unity of a multicellular organism. Those details of the genetic programme, which become materialized in the single cells seem to contain the code for the quality, yet in all probability not for the quantity, of the materials produced by the given cell. Thus *the interacting cells have to "learn" the potentials of the interacting partner during a critical stage of ontogenesis.* Imprinting probably plays a role in this "learning" process, by disclosing for the target cell the properties of the signal emitter cell, to which the target cell is obliged to adapt the capacity of its signal receiving system. The down-regulation mechanism required for arresting the action of a given hormone is probably only latently present, if at all, in the prenatal period, because it takes effect only postnatally [42], at the primary interaction between the hormone and its target cell. Hormone peaks appear postnatally in succession, and the peaks of the related hormones are separated from one another by time intervals long enough to permit the establishment of receptor specificity as well as the fine tuning of the target cell for reception of the signals emitted by the regulating cell.

The regulation of the whole organism takes place within a hierarchical system. The different elements of the endocrine system are variously interrelated, and also operate in a hierarchical order. The pituitary gland, which is at the top of the hierarchy, is controlled by the nervous system. The adaptation of the systems to one another is a necessary prelude to their co-ordinated functioning, and takes place in all probability prenatally. A characteristic example of perinatal adaptation is the sexual imprinting, during which the hypothalamic rather than the hypophyseal nuclei are respond to the sexual steroids (this does not, naturally, exclude the perinatal adaptation of the pituitary gland as well). Adaptation also involves a "calibration" of the components involved in the interaction (This is not surprising, if it is taken into consideration that e.g. the cells of same individual are responsible for antigenicity and immunity, yet they have to adapt to one another to be able to suppress auto-immune response. Although the antibody producing and antigen-like cells are controlled by the gene complement of the same organism, no suppression occurs without presence of the auto-antigen).

It follows from the foregoing considerations that presence of certain (foreign) materials during imprinting (tuning or fine-tuning) may prevent the integration of the different micro-systems and thereby the development of the macro-system as well. Alteration of a single link in the interacting systems may affect the macro-system to the extent that even *a positive local change may have a negative impact* on it. It should also be noted that the tuning effected by abnormal imprinting does not necessarily, or not exclusively, take place in the adequate system. Perinatal treatment with sexual steroids interferes with the normal course of glucocorticoid receptor maturation, too [21], and neonatal glucocorticoid exposure also effect the ouabain receptor [43]. This can be explained by the structural similarity of the imprinters, or by a disturbance in the macro-system, which may also change the trend of imprinting. As already noted, the non-specific stimulus conveyed by maternal stress may adversely affect the sexual imprinting of

the foetus (homo-or bisexuality) [8].

Interrelationship between imprinting and external influence

All recognition systems are open systems, but the degree and duration of openness varies with the nature of the system. The nervous system, with its life-long capability of "learning" is open for lifetime. In contrast, behavioural imprinting is limited to a critical perinatal period, and so is basic immunological imprinting, although the immune system would "learn" as long as it exists. However, behavioural imprinting may return later in life for short critical periods (e.g. imprinting of the maternal instinct occurs around hatching or parturition), whereas immunological imprinting for the differentiation of "self" from "non-self" terminates perinatally. Sexual as well as hormonal and enzyme imprinting, too, takes place and terminates perinatally, but while sexual imprinting is finalized perinatally, hormonal imprinting continues to occur in the cytogenetic organs for life-time, and once imprinted cells may become re-imprinted in the conditions of de-differentiation, e.g. during regeneration or in culture (in vitro). It appears that periodic availability for imprinting is a common specific property of recognition system, and depends on the nature of the systems's function.

Why does imprinting take place perinatally?

Although imprinting can, in certain cases, take place later and earlier in life, its intimate association with the perinatal period, which seems to be physiological as well as deterministic, invites explanation. This seems to be easy enough in the case of sexual imprinting, since the adjustment of the hypothalamic nuclei presuppose the segregation of the foetal and maternal organism, i.e. exclusion of the influence of maternal and placental hormones. The excess of the latter relative to the foetal hormones would prevent the male trend of sexual development. A similar explanation may be offered for the association of hormonal imprinting with the perinatal period. Apart from a major interference of maternal hormones, the receptors of the foetal organism cannot mature in utero, owing to subordination of foetal responses to the selective dominance of the placenta, and to other aspects of foetal development. Moreover, neonatal hormone production differs from the foetal one both qualitatively and quantitatively, owing at least partly to the change of environment. The impact of environmental changes could also accunt for the perinatal timing of enzyme imprinting. It is, in all probability, necessary that the neonate's hormone (substrate) production is recognized as "self", at least in respect of its quantitatie relations, which determine the number of receptors required for a normal functioning of the receptor—hormone relationship in the independent organism.

Immunological imprinting, i.e. the ability to differentiate between "self" and "non-self", is acquired in a long process which commences with the maturation of the immune system and terminates in the early postnatal period, at different times depending on the species and on the nature of the antigen. Since the iso-enzymes (proteins) appearing in the different stages of development are slightly dissimilar, there is reason to postulate that the proteins aquire their immunologically "safe" final form only after the unfolding of the recognition potentials of the immune system. Exceptions are, of course, those protein structures, which appear later in life (ocular lens, male gametes); a delay of the development of the basic recognition mechanism until then would seriously affect the balance between the organism and its environment (homeostasis).

Imprinting and disposition

Summarizing the foregoning considerations, the implication lies close at hand that the perinatal interactions between future target cells and environmental chemical (key) stimuli represent essentially a cellular-level *chemical imprinting*, which plays a fundamental role in the coordination of the recognition mechanisms of the organism. The different forms of cellular-level imprinting are basically involved in the adjustment of endogenous functions, whereas behavioural imprinting covers the fixing of those early environmental stimuli, which are fundamentally important for the survival of the individual. Yet, chemical imprinting also plays a decisive role in the establishment of

behavioural patterns, not only because the nervous responses are originated at receptor level and are mediated chemically, but also because many important environmental signals (food preference, impact of cholesterol diet on cholesterol homeostasis in adulthood etc.) are interpreted by the organisms in terms of chemical signals.

The perinatal timing of chemical imprinting easily gives the impression that the events covered by it are genetically determined. Prior to the ingenious discoveries of Burnet and Medawar, immunobiologists generally believed that immunity was developed exclusively against "foreign" antigens, and there was no presumption that "self" —antigens, too, had to be neutralized in a certain period of ontogenetic development. The recognition of immune suppression stemmed from the clonal selection theory and from experimental evidence of actively acquired tolerance. The textbooks still state [44] that individual variations in the operation of the microsomal enzyme system are genetically determined, although the variations could as well be attributed to individual differences in neonatal imprinting. Faulty hormonal imprinting in the neonatal age has the same result as the gene-level defect of hormone receptors, and the behavioural abnormalities of a child deprived of parents or brought up in an unsocial environment may be similar to those conferred by gene-level defects. If follows that *current conceptions on disposition should be modified, inasmuch as further to the hereditary aspect they should also cover the possibility of perinatal imprinting,* whose impact may be, in given conditions, equivalent to that of the hereditary predisposition.

In this light it is indeed difficult to differentiate between the issues of perinatal imprinting and genetic determination, regardless whether the functions or traits in question are normal or abnormal. Although the greater part of the congenital defects is genetically determined, a minor part is consequent upon intrauterine damages. The apparent stupidity, unsocial or even antisocial behaviour of a child on entering the school may as well be consequent upon hereditary traits as upon negative influences during upbringing, i.e. upon faulty behavioural imprinting in early childhood [45, 46]. However, the better understanding of the role of hereditary and imprinting-related factors requires a sharp differentiation between in utero effects (including the genetic factors) and (perinatal) imprinting—associated effects. *Imprinting involves the joint effect of endogenous factors and exogenous influences, because part of the normal genetic programme required collaboration of the environment for running* to its end. *Faulty imprinting* has at the same time a *teratogenic* effect, regardless whether it occurs pre or postnatally.

The process of imprinting is similar to, but not identical with, learning. *Learning* is a special potential of the nervous system and immune system, which can take effect at any time in life, being *not limited to a critical period* (or periods). Although both systems "remember" what they have learned, the information so acquired can be deleted from the nervous system, or its "memory" fades away sponteneously if repetitions are lacking. *Imprinting,* on the other hand, *can take place* in both systems *exclusively within a critical period, by activation of the genetic programme, and the information so acquired persists for lifetime.* While the learning process of the nervous system is repeatable, imprinting is irrepeatable, and if it fails to take place in the critical period, the organisms misses it lifelong, or even fails to survive.

Hereditary transmission of the information conveyed by imprinting

It was demonstrated experimentally that rats neonatally treated with thyroxin for a few days were in adulthood less responsive to thyrotropic hormone (TSH) than the untreated controls [10]. This shows that, for lack of imprinting by TSH in the critical period, the thyroidic TSH-receptors became insensitive to the adequate hormone. Neonatal exposure to thyroxin gave additionally rise to certain hypothalamic-hypophyseal, thyroidic and gonadal abnormalities [47, 48]. Mating of female rats so treated to untreated males caused for functional disturbances of the thyroid and gonads in the first offspring generation. The functional abnormalities persisted to a certain extent also in the second offspring generation. Suckling rats given alloxan showed a decreased glucose tolerance in adulthood. The induced diabetes

persisted latently in six offspring generations, and become clinically expressed in the seventh generation [49]. A single neonatal treatment of rats with insulin altered the binding capacity of the hepatic insulin receptors. The first offspring generation responded by an even greater change to perinatal insulin exposure [50], but no such effect could be observed in the further generations. It follows that although the impact of the external influences acting on the organism in the critical perinatal period is not comparable to that of the gene-level effects, it does outlast the parent generation directly involved in imprinting, to judge from its reappearance in one to more offspring generations. Although it is still obscure, whether transmission to the offspring occurs directly via membrane level changes, or indirectly, by a potential for adequate response to imprinting-like environmental influences, the great variety of the factors involved in chemical imprinting and the hazards involved in the current trends of chemization should at least inspire speculations on new approaches to the problem.

The mechanism of the cell-to-cell transmission of imprinting

Imprinting takes place perinatally, when a hormone, a substrate, an antigen or an individual meets the signal receiver, which initiates the process. Since, however, the cells of an adult animal represent the distant progeny of those which had been directly involved in imprinting, a cell-to-cell transmission of the information is self-evident. The only exception is the nervous system, whose cells are not exchanged by proliferation. As to the cells of the immune system, it is known that those capable of recognizing the auto-antigens are permanentely suppressed, but suppression presupposes partly the permanent presence of the auto-antigen, partly the permanent activity of the suppressor T-cells, which act on the "forbidden" clones. The other cells of the immune system proliferate continuously, and transmit the gene-level information pertaining to the recognition of "foreign" molecules from one generation to the other.

While the transmission of the information conveyed by imprinting plays no role in the case of the nerve cells and certain immune cells, it is vital for hormone receptors and enzymes [51], and presupposes the operation of either a gene-level or a membrane-level mechanism. The prerequisite of membrane-level transmission would be a self-reproduction of the membrane receptors, viz. of cytosolic enzymes, which takes place indenpendently of the genetic programme. This hypothesis is supported by certain implications [52], but evidence remains to be obtained for its substantiation. A third alternative is the cell-cell transmission of the *information,* by involvement of an as yet unknown material substrate. There is evidence that mammalian cells transmit the information conveyed by imprinting to daughter cells by direct contact [19], and unicellulars transmit it in culture by means of a transmission factor released into the nutrient medium [53]. It cannot be excluded either that imprinting takes effect by readjusting the genetic programme (by acting on a regulator locus?) in the perinatal period, when cellular hormone internalization increases several times over the adult level, and the final issue is a reassembly of the gene complement (such as known in the immune system in connection with the great variety of antigens), which persists in the offspring generations.

CONCLUSIONS: CONGENITAL, HEREDITARY AND ACQUIRED TRAITS

The conception of congenital abnormality has been currently modified. While formerly it covered mainly morphological defects, the current interpretation also covers biochemical defects, which are in fact more numerous and also more serious in part of the cases. Thus not only the defects obvious at birth are regarded as congenital, but also those which become expressed later in life, in childhood or adulthood. Nonetheless, it is still, extremely difficult to differentiate genetic from environmental impact on an abnormality regarded as "congenital", i.e. to distinguish the responsibility of genuine genetic determination from that of perinatal influences of teratogenic importance for the given defect.

The segregation of the peri- and postnatal period of imprinting is of decisive importance, for

the developing foetus is obliged to adapt itself to the maternal organism and it is exposed to the effect of maternal sentiments like oppression, fear or joy already in utero. The maternal organism also plays a role in direct chemical imprinting, to judge from the child's greater immunological proximity to the mother than to the father, despite the equal share of both parents in the genetic material of the offspring. During the maturation period of the foetal immune systme, the maternal proteins increasingly gain access to the embryo through the placenta (which tends to become less selective in advanced gestation), and may impress the developing immune system as "self" which can never occur with paternal proteins. Maternal influence on chemical imprinting continues in the period of lactation, during which the maternal proteins present in the milk are absorbed though the still immature intestinal mucosa of the neonate. The maternal hormones appearing in mother's milk probably also play a role in the imprinting of the infant, in other words in tuning the neonatal organism to the maternal "wavelegths". A similar maternal impact on enzyme imprinting could as well be postulated. The role of the mother in behavioural imprinting is generally known.

The foregoing considerations support the hypothesis that *the predisposing role of the imprinting-associated events may be equivalent to that of genetic predisposition* in respect of certain pathological conditions of the systems requiring the collaboration of imprinting for development. The microsomal enzyme system or the hormone system develop not only by a genetically determined programme, but also by perinatal imprinting, which may in both cases lead to, or permit, certain pathological alterations, if it takes a faulty course.

In this light, alcohol or drug addiction, too, may be consequent upon imprinting as well as on genetic determination, and it seems even more feasible that *diseases arise by the interaction of endogenous and exogenous factors. A system, which is labile already at the gene-level, will become disased if it suffers a faulty imprinting, but will stay labile, yet healthy, if the imprinting is correct. A genetically stable system, in contrast, will be pushed toward the physiological limits of its normal function, but not beyond these, by faulty imprinting. The collaboration of deterministic endogenous factors and changed environmental influences is therefore of decisive importance, and should be taken into consideration not only for diagnostic approach to a disease, but also for contemplation of a perinatal intervention.*

Genetic demages tend to become more frequent recently, not only because they are more easily recognized, but also because environmental mutagens are more numerous than formerly. The relative importance of disturbances in imprinting is, however, still greater. Pre or perinatal exposure to environmental chemicals is greater than ever. The mother deals with scores of synthetic compounds during both the early and late stages of her pregnancy. Some of these chemical are teratogenic in early pregnancy, and account for faulty imprinting in the advanced state. The chemicals used at delivery act on the neonate in the most critical stage of imprinting, and the pharmacons taken by the nusing mother, or those present in the cow's milk given to artificially fed babies, represent adverse environmental influences for the neonate. *Perinatal interventions may bias the normal course of imprinting, exactly as exposure to a foreign antigen in the period critical for the differentiation of "self" from "non-self" will lead to later acceptance of the foreign molecule as self.* The insight already obtained into immunological disturbances arising from this cause reminds that similar hazards may threaten the development of other recognition systems, too.

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